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Aromatherapy on a large scale: exposing entire adult holding rooms to ginger root oil increases the mating competitiveness of sterile males of the Mediterranean fruit fly in field cage trials

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Abstract

The sterile insect technique (SIT) is widely used in integrated programs against fruit fly pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Unfortunately, the mass-rearing procedures inherent to the SIT often lead to a reduction in male mating competitiveness. One potential solution involves the pre-release exposure of males to specific attractants. In particular, male exposure to ginger root oil [*Zingiber officinale* Roscoe (Zingiberaceae); hereafter GRO] has been shown to increase mating success dramatically in field cage trials. Initial studies exposed small groups of males (25 individuals), but more recent work has demonstrated that GRO exposure involving standard storage boxes (containing ≈ 36 000 males) also results in enhanced mating performance. The objective of the present study was to determine whether aromatization of entire trailers, holding ≈ 14 million sterile males from a genetic sexing [temperature sensitive lethal (*tsl*)] strain, increases male mating success. Independent of the total dose, spatial distribution, or type of dispenser used, sterile males exposed to GRO for a 24-h period displayed greater mating success than non-exposed males in mating cage trials (in which *tsl* males competed against males from a standard, bisexual strain for females from this same standard strain). Averaged over all experiments, *tsl* males exposed to GRO obtained 54% of all matings compared to 38% for non-exposed *tsl* males, an increase of 42%. The implications of these findings for SIT programs against *C. capitata* are discussed.

Introduction

The sterile insect technique (SIT) is an environmentally benign approach to suppress or eradicate insect pests and is widely used in integrated programs against tephritid fruit fly pests, particularly the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Hendrichs et al., 2002; Klassen, 2005). The technique involves mass production, irradiation (sterilization), and release of males of the target species into the environment. Matings between sterile males and wild females yield infertile eggs, which reduces the reproductive potential of the wild population. Thus, the success of the SIT depends, to a large extent, on the ability of released, sterile males to attract and copulate with wild females. This behavioral capability is especially important for species, such as *C. capitata*, characterized by 'complex' mating behavior (Lance & McInnis, 2005) in which males produce multiple sexual signals using various modalities (visual, acoustic, and olfactory), and females display a high degree of mate selection based apparently on male courtship performance (Whittier et al., 1992, 1994).

Unfortunately, the mass-rearing procedures inherent in the SIT often lead to a reduction in the mating competitiveness of released medfly males, and sterile males typically have low mating success relative to wild males (Roessler, 1975; Shelly et al., 1994; Lance et al., 2000).

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Consequently, an important and persistent problem for the SIT is the development of simple and inexpensive means to enhance the mating performance of released sterile males. A series of experiments (Shelly et al., 2005 and references therein) identify a potentially productive approach involving the pre-release exposure of sterile males to specific chemical attractants. In particular, experiments involving different strains tested under different environmental conditions have demonstrated that exposure to the aroma of ginger root oil [*Zingiber officinale* Roscoe (Zingiberaceae); hereafter GRO], containing the known attractant α-copaene (Flath et al., 1994a,b; Nishida et al., 2000), significantly increases the mating success of sterile males of *C. capitata*.

Following initial demonstration of this phenomenon, research has explored the possibility of its implementation in large-scale medfly SIT programs by progressively increasing the rigor of the testing procedure and the scale of male exposure. Measurements of mating success have been conducted under increasingly natural conditions. Initially, short-term (4 h) tests were conducted in small ('single tree') field cages (3 m high, 2.5 m diameter; Shelly, 2001; Shelly et al., 2004). More recently, however, we have measured mating performance over several days in large field enclosures ($16 \times 6 \times 2.5$ m) containing >10 host trees (Shelly et al., 2005) or in the open field, specifically coffee fields on Kauai, Hawaii (Shelly et al., 2007). Likewise, we have increased the scale of exposure. Initially, GRO exposure was performed on small groups of males (25 individuals) held in small cups (400 ml; Shelly, 2001). Subsequently, increased mating success was reported after GRO exposure involving ≈ 36 000 males held in individual storage boxes (Plastic Adult Rearing Containers or PARC boxes, $0.60 \times 0.48 \times 0.33$ m; Shelly et al., 2004) and ≈ 1.25 million males held in emergence towers ($0.7 \times 0.7 \times 1.5$ m; Shelly et al., 2006). While the relative strength of the effect has varied among the different studies, GRO exposure of adult male medflies has consistently increased their mating success independently of the exposure regime or assessment method.

The present study examines the impact of GRO exposure at a larger spatial scale and specifically tests whether aromatization of entire trailers, holding several hundred PARC boxes each, increases male mating success. The study was conducted at the David Rumsey Sterile Fruit Fly Eclosion Facility, Medfly Preventative Release Program, Los Alamitos, CA, USA. Started in 1996, this program makes aerial releases of sterile males over an area of ca. 6400 km^2 that includes the Los Angeles basin and surrounding areas. Approximately 40–45 million sterile males (from ca. 1000–1200 PARC boxes) are released daily. As described below, PARC boxes containing pupae, and subsequently eclosed adults, are stored in holding trailers prior to release, with each trailer holding ca. 360 PARC boxes or ca. 14 million sterile males. Here, we describe the results of mating trials conducted in field cages that compare the relative mating success of sterile males from GRO-aromatized trailers vs. non-aromatized trailers.

Materials and methods

Operating procedures at the Los Alamitos facility

Knowledge of the daily operations at the fly emergence facility is essential to understanding the experimental protocol adopted, and here we provide a brief summary of the processing cycle. The facility receives medfly pupae via air shipments from mass-rearing facilities in Guatemala and Hawaii. Within hours of delivery, the pupae are transferred to paper bags (110 ml pupae per bag, 1 ml ≈ 60 pupae), and six bags are placed in individual PARC boxes (i.e., each box receives ≈ 39 600 pupae). Adult food (sugar agar gel) is then placed on the screened opening on the top of the PARC boxes, and the boxes are transferred to the storage trailers ($17.8 \times 3.1 \times 2.4$ m high, volume ≈ 132 m^3). The trailers used in this study contained 350–408 PARC boxes; these were stacked on top of one another in groups of six, and the stacks were then arranged in 15–17 rows (four stacks per row) oriented perpendicularly to the long axis of the trailer. Trailers were maintained at 26 ± 1 °C and 50–65% r.h. with lights continuously off. At one-third and two-thirds the total length of the trailers, two large vertical fans were mounted on boards to provide ventilation; all four fans in a trailer blew air in the same 'lengthwise' direction. Peak emergence of the adult males occurs 2 days after pupal arrival and placement, and after another 2 days males are chilled (at 4 °C for 60 min, a procedure called 'knockdown'), transported to an air strip, and released by small aircraft. Thus, in a processing cycle, flies from a given shipment are held for 5 days: pupae arrive and are placed in the PARC boxes on day 1, peak adult emergence occurs on day 3, and the flies are chilled and released on day 5. Between removal of flies for knockdown and re-filling with new pupae, trailer interiors are rinsed with water and then aerated and dried by leaving two doors open for a day.

Study insects

Although from two different locations, the pupae arriving at Los Alamitos were from the same genetic-sexing strain (Vienna-7/Tol-99), which possesses a sex-linked temperature sensitive lethal [*tsl*] mutation allowing selective elimination of females at the egg stage (Franz et al., 1996) (i.e., all of the arriving pupae were male). Because the number of pupae arriving from Guatemala far exceeded that arriving from Hawaii, we used *tsl* males only from Guatemala in our study. The Guatemalan flies were produced in the

USDA-Moscamed facility at El Pino, the largest such facility in the world, with an output of ca. 3 billion pupae per week. Pupae were dyed (fluorescent orange-red) and irradiated 48 h before emergence under hypoxia at 145 Gy using a Gammacell 220 (MDS Nordion, Ottawa, Canada) with Co^{60}.

Because wild flies were not present in California and their importation for experimental purposes was prohibited, we used males and females from bisexual strains reared in Guatemala (for tests conducted in 2004) or Hawaii (for tests conducted in 2005). Use of a different strain in 2005 allowed evaluation of the robustness of the results obtained in the previous year. The Guatemalan ('Petapa') strain has been maintained in the laboratory for ca. 20 years as a small colony (several thousand breeding adults per generation) and receives regular infusions of wild flies. The Hawaiian ('Maui') strain was established in 1993, mass-produced from 1995 to 2002, and since 2002 has been maintained as a small colony at the USDA-ARS facility in Honolulu, HI, USA. During the study, irradiated (at the same dose used for the *tsl* strain) and dyed (fluorescent green) pupae from the Petapa or Maui strains were shipped 2–3 times per week along with the regular shipments of *tsl* pupae. Adults were separated by sex within 24 h of emergence and maintained in 5 l plastic screen-covered buckets (100–150 flies per bucket). Males were fed the same sugar agar gel as the *tsl* males, and females were fed the sugar agar gel plus a sugar-protein (yeast hydrolysate) mixture (3 : 1, vol/vol). Petapa and Maui flies were held in the laboratory at 25 ± 2 °C and 70% r.h. under a L14:D10 photoperiod.

GRO exposure

Ginger root oil, which was obtained from Citrus and Allied Essences Ltd. (Lake Success, NY, USA), contains α-copaene (a hydrocarbon sesquiterpene) in low concentration (0.4%; S Young, pers. comm.) with the positive enantiomer predominating (81%, Takeoka et al., 1990). GRO contains additional sesquiterpenes, but their effect on *C. capitata* either independently or in combination with α-copaene remain largely unknown (but see Flath et al., 1994a,b).

Over the course of our project, conducted during August–September in 2004 and 2005, we varied the dose, spatial distribution, and delivery 'system' of the GRO (as noted above, we also used two different strains as substitutes for wild flies). Here, we first describe methods common to all exposure regimes and then those particular to 2004 and 2005, respectively.

Common methods. For all experiments except one (final experiment, 2005; see below), we ran four mating trials (two with treated *tsl* males and two with control *tsl* males) on a given day. Test days were operationally paired, such that the trials conducted on two consecutive days used *tsl* males from the same two trailers, one exposed to GRO (yielding treated males), and one not exposed to GRO (yielding control males). To obtain treated and control *tsl* males for testing, we randomly selected (and marked with flagging tape) four PARC boxes each in trailers holding treated or control *tsl* males. Box selection and tagging was performed on day 3 of the processing cycle, and the GRO was placed in the treated trailers at 06:00 hours on day 4 of the cycle (i.e., 1 day before knockdown). Shelly (2001) showed that GRO exposure to immature (1-day-old) males enhanced their mating performance (when tested 7 days later as mature adults). Thus, the timing of GRO exposure employed here was considered appropriate. The GRO was removed 24 h later, and immediately afterward the PARC boxes from both treated and control trailers were moved into refrigerated trailers for knockdown between 06:00 and 07:00 hours. The flagged boxes (four treated and four control) were set aside during the knockdown, at the end of which we collected samples of 100–200 males from each of the selected eight boxes. Treated and *tsl* control males were transferred to plastic buckets, which were placed in separate rooms, and provided sugar agar gel. These flies were held under the same laboratory conditions noted above for the Petapa and Maui flies. Males were held until testing 1 or 2 days later (i.e., when the majority of *tsl* males were 3 or 4 days old, respectively), except in one experiment in which *tsl* males were held 4 or 5 days after knockdown to assess the effect of GRO exposure over a longer-time interval.

GRO exposure regimes–2004. Trailers were re-used over the study period, but trailers that received GRO were never used as control trailers. In addition, among the trailers receiving GRO, 2–3 weeks typically elapsed between successive applications of GRO during which time the trailers were used as part of routine operations. In 2004, treated *tsl* males were collected from eight different trailers, and control *tsl* males were taken from seven different trailers.

Extrapolating from preliminary work conducted in Hawaii, we estimated that 9 ml of GRO per trailer would be sufficient to increase the mating success of the sterile *tsl* males. Given the rectangular shape of the trailers, we initially used a 9-ml dose of GRO distributed among 18 points (sources) distributed evenly over the length of the trailer (one source between adjacent rows with extra sources placed between rows adjacent to the fans). At each source, we placed 0.5 ml of GRO on a cotton wick (2.5 cm length, 1 cm diameter) fitted inside a small, perforated plastic basket (the same type used to hold lures in Jackson traps), which, in turn, was suspended from a wire hook between adjacent PARC boxes. All wicks were suspended at

mid-height of a stack (i.e., from the top of the third box from the floor in the stack of six boxes).

As this exposure regime appeared to boost mating success, we used the same number and distribution of GRO sources in two additional exposure regimes but increased the dose per source to 1.0 and 2.0 ml, respectively (i.e., for total doses of 18 and 36 ml, respectively). For the 1.0 ml dose/source, we used the same-sized wick as above, and for the 2.0 ml dose/source we applied 1.0 ml to each of two wicks, which were placed in two baskets suspended from the same wire hook. In the final exposure regime tested in 2004, we distributed a total dose of 36 ml of GRO among only four sources, two at each of the two fan 'banks' in a trailer. At each source, we applied 3 ml of GRO to each of three wicks (length 5 cm × 1 cm in diameter) resting in an aluminum foil-lined Petri dish, which, in turn, was placed on the wooden structure used to support the fans. Thus, two Petri dishes, each containing 9 ml of GRO were present at each fan bank, and the dishes rested next to the fans separated by about 1 m at a height of approximately 1 m.

GRO exposure regimes–2005. Because the Los Alamitos facility introduced GRO exposure as part of their standard operating procedure in January 2005, there were only two trailers at the facility that were not used routinely and hence that had never been exposed to GRO by the summer of 2005. Consequently, all control *tsl* males were taken from these two trailers, which housed only our control flies and no flies destined for release (i.e., only four PARC boxes as opposed to 350–408 PARC boxes; temperature and humidity were maintained at normal levels). Treated *tsl* males were taken from 15 trailers that had received multiple GRO exposures. Trailers were re-used at least 7 days after the previous GRO exposure. As noted above, we replaced the Petapa strain with the Maui strain in 2005 to assess whether the results obtained were consistent with those obtained in 2004.

In 2005, we first repeated the final experiment of 2004 (i.e., 36 ml of GRO distributed among four sources placed at the fan banks). We then repeated this experiment but halved the dose (i.e., 2.25 ml of GRO was applied to two wicks per source for a total of 9 ml of GRO per fan bank or 18 ml of GRO per trailer). In the final two experiments of 2005, we replaced wicks with silicon panels, where each panel (20 × 8 × 0.15 cm thick) contained 10 g of GRO (specific gravity of GRO = 0.88, so 10 g ≈ 11.4 ml). Although more expensive than cotton wicks, the panels were easier to handle and thus warranted testing. For exposure, we suspended two panels from each fan bank using a metal hanger. As with the wicks, the panels were positioned next to the fans about 1 m apart and 1 m off the floor. Using this same exposure regime, we performed two sets of mating trials. The first was conducted following the procedure used for all preceding experiments, that is, four mating tents (two with treated *tsl* males and two with control *tsl* males) were conducted per day. In the other set, we ran three mating trials per day, one having *tsl* males exposed to the GRO-laden panels, one having *tsl* males exposed to 36 ml of GRO distributed among four (wick) sources, and one having control *tsl* males. Correspondingly, for this final set of mating trials, we selected and marked two PARC boxes (and not four) per trailer for a total of six (and not eight) marked PARC boxes per knockdown. All other aspects of fly handling were identical to the other experiments.

Mating trials

Four nylon-mesh, field tents (3 m diameter × 2.5 m high) were set up in a vacant lot on the grounds of the Los Alamitos facility. Excepting the final experiment of 2005, we ran four tents per test day, two containing treated *tsl* males and two containing control *tsl* males. *Tsl* males from each of the selected PARC boxes were used in only one field tent, and thus *tsl* males from four different PARC boxes (two treated and two control) were used on a given day. In the final set of mating trials of 2005, we ran three mating tents per day, representing *tsl* males from three different PARC boxes (two treated and one control) as described above.

In all experiments, groups of 75 males from the bisexual strain (Petapa in 2004, Maui in 2005), 75 females from the bisexual strain, and 75 treated *tsl* males or 75 control *tsl* males were released in each tent between 08:30 and 09:30 hours (males were released 15 min before females). Tents were covered with a shade cloth to reduce insolation and contained two artificial trees (2 m tall containing ≈ 450 leaves resembling those of *Ficus benjamina* L.). Artificial trees were used, because they provided a chemically neutral substrate on which the flies displayed the entire complement of natural activities. Mating pairs were collected in vials for 4 h after release and chilled in a freezer. Males were then identified using an ultraviolet (black) light to determine dye color (orange-red = *tsl* male; green = bisexual male). Unmated flies were removed from the field cages following completion of a trial. Assignment of treated or control *tsl* males to specific tents was alternated between successive test days. Air temperatures were recorded at the start and stop of the mating trials, and these averaged 22.3 °C (range: 20.0–24.7 °C) and 26.7 °C (range: 24.4–27.8 °C), respectively (data over both years, n = 24 [2004] +30 [2005] = 54 days).

Statistical analyses

The Student's t-test and ANOVA were used for pair-wise and multiple comparisons, respectively, as assumptions of

Table 1 For 2004, absolute mating success of GRO-exposed (treated) and non-exposed (control) *tsl Ceratitis capitata* males in field-cage tests conducted 1–2 days post-knockdown. Average (±1 SE) numbers of matings are given for n replicates; t-values refer to comparisons between *tsl* and Petapa males for a particular GRO exposure regime

Experiment	GRO dose (ml/trailer)	GRO sources (no./trailer)	Male type	Number of matings	n	t-value
1	9	18	Treated *tsl*	21.0 (2.4)	10	0.4 ns
			Petapa	19.8 (1.1)		
	None		Control *tsl*	13.0 (1.7)	10	3.5**
			Petapa	23.5 (2.4)		
2	18	18	Treated *tsl*	26.0 (1.3)	14	0.1 ns
			Petapa	26.2 (1.6)		
	None		Control *tsl*	15.9 (1.2)	14	6.6***
			Petapa	30.5 (1.9)		
3	36	18	Treated *tsl*	22.9 (1.6)	10	1.4 ns
			Petapa	19.9 (1.4)		
	None		Control *tsl*	10.3 (0.7)	10	7.0***
			Petapa	25.2 (2.0)		
4	36	4	Treated *tsl*	23.3 (1.8)	14	1.6 ns
			Petapa	18.7 (2.3)		
	None		Control *tsl*	13.2 (1.2)	14	3.2**
			Petapa	23.1 (2.8)		

ns, not significant (P>0.05); **P<0.01; ***P<0.001.

normality and equal variances were met in all cases. Proportions were arcsine transformed for all tests.

Results

Year 2004

Results from 2004 showed that all GRO exposure regimes resulted in a significant increase in the mating success of treated *tsl* males (Table 1). In all experiments conducted 1–2 days after knockdown, Petapa males obtained significantly more matings per replicate than control *tsl* males, whereas no significant difference in absolute mating frequency was detected between Petapa and treated *tsl* males.

The impact of GRO on mating success is clearly illustrated in comparing the relative success of treated and control *tsl* males (Figure 1). These data revealed that, in all experiments, treated *tsl* males obtained a significantly greater proportion of the total matings than control *tsl* males (50–57% vs. 30–39%, respectively). In addition, there was no apparent difference in the relative mating success of treated *tsl* males among the different GRO exposure regimes (ANOVA: $F_{3,44} = 1.6$, $P = 0.20$). As would be expected, control *tsl* males also displayed similar mating success across experiments ($F_{3,44} = 1.1$, $P = 0.32$). Based on data from all four experiments, treated *tsl* males achieved, on average, 53% (±9.6; n = 48) of the total matings per replicate compared to 35% (±11.3; n = 48) for control *tsl* males (t = 8.3, P<0.001).

The results from the single experiment in which trials were conducted 4–5 days after knockdown (and exposure to 18 sources with 1.0 ml GRO per source) were similar to those reported above. In this experiment, Petapa males achieved 22.5 (± 1.6) matings per replicate (n = 8) compared to only 16.1 for control *tsl* males (t = 3.1, P<0.01), but no difference was evident between Petapa (20.0 ± 1.4) and treated *tsl* (21.1 ± 1.1) males (t = 0.6, P>0.05).

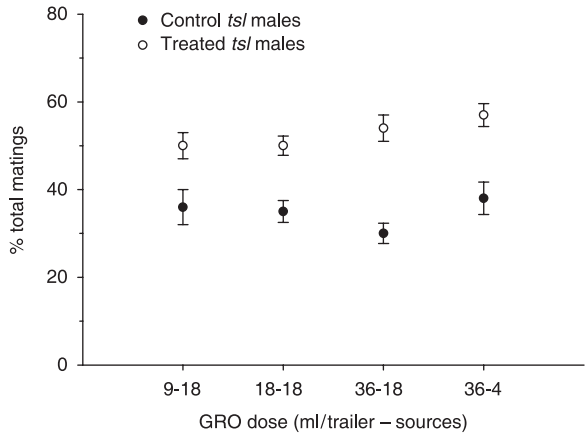

Figure 1 Relative mating success (percentage total matings ± SE) of treated and control *tsl Ceratitis capitata* males for the different GRO exposure regimes investigated in 2004 (trials conducted 1–2 days after knockdown).

Table 2 For 2005, absolute mating success of GRO-exposed (treated) and non-exposed (control) *tsl Ceratitis capitata* males in field-cage tests conducted 1–2 days post-knockdown. Average (±1 SE) numbers of matings are given for n replicates; t-values refer to comparisons between *tsl* and Maui males for a particular GRO exposure regime

Experiment	GRO dose (ml/trailer)	GRO sources (no./trailer)	Male type	Number of matings	n	t-value
5	36	4	Treated *tsl*	19.5 (1.0)	10	1.2 ns
			Maui	17.1 (1.6)		
	None		Control *tsl*	17.3 (1.9)	10	2.9**
			Maui	27.9 (3.1)		
6	18	4	Treated *tsl*	22.2 (1.4)	10	0.8 ns
			Maui	24.3 (2.1)		
	None		Control *tsl*	18.7 (2.3)	10	1.6 ns
			Maui	24.0 (2.6)		
7	45 (panels)[1]	4	Treated *tsl*	28.9 (1.6)	16	5.4***
			Maui	17.4 (1.4)		
	None		Control *tsl*	22.1 (2.2)	16	0.5 ns
			Maui	23.6 (2.5)		
8	36	4	Treated *tsl*	23.3 (2.0)	12	0.7 ns
			Maui	21.2 (2.0)		
	45 (panels)	4	Treated *tsl*	26.7 (1.4)	12	1.5 ns
			Maui	21.5 (1.6)		
	None		Control *tsl*	16.7 (1.3)	12	4.5***
			Maui	26.8 (1.8)		

[1]10 g GRO/panel, where 1 g ≈ 1.14 ml.
ns, not significant (P>0.05); **P<0.01; ***P<0.001.

Correspondingly, treated *tsl* males were found to obtain a significantly higher proportion of the total matings than control *tsl* males (51 vs. 42%, t = 2.4, P<0.05). Following exposure to 18 sources with 1.0 ml of GRO per source, treated *tsl* males held 4–5 days post-knockdown accounted for a similar proportion of the total matings as treated *tsl* males held only 1–2 days post-knockdown (51 vs. 50%, t = 0.4, P>0.05).

Year 2005

The results obtained using Maui males were similar to those observed for the Petapa males for the same exposure regime (36 ml of GRO at four sources) in the preceding year, that is, treated *tsl* males had similar mating success as Maui males, but control *tsl* males were competitively inferior to Maui males (Table 2, Figure 2). Comparing relative mating frequencies for this exposure regime, Maui and Petapa males did not differ significantly in their performance against control (Maui −61% total matings, n = 10; Petapa −62%, n = 14) or treated (Maui −46%, n = 10; Petapa −43%, n = 14) *tsl* males (t-test, P>0.05 in both cases). Based on these data, we consider that Maui and Petapa males are equivalent competitors relative to the *tsl* males and that results obtained for one of these strains are applicable to the other.

Although a total dose of 18 ml of GRO positively affected mating performance when distributed among 18 sources (Table 1), this same dose had no detectable effect when distributed among only four sources, presumably

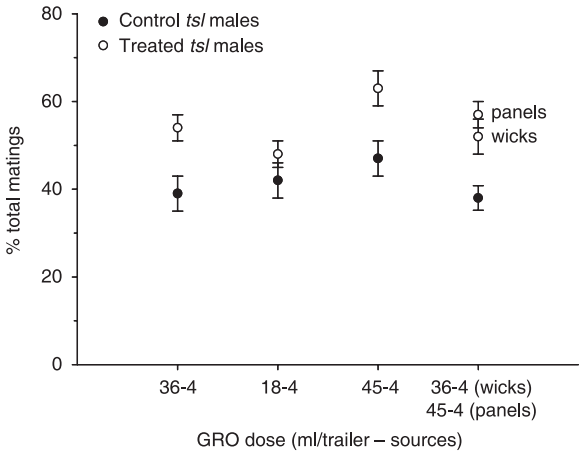

Figure 2 Relative mating success (percentage total matings ± SE) of treated and control *tsl Ceratitis capitata* males for the different GRO exposure regimes investigated in 2005. In contrast to all other experiments, in the final experiment of 2005 two types of treated males (wick- and panel-exposed) were tested over the same days.

because of uneven or inadequate spread of the GRO aroma (Table 2). Maui males obtained significantly more matings per replicate than treated or control *tsl* males, and a comparison of relative mating success revealed no significant difference between treated and control *tsl* males ($t = 1.1$, $P>0.05$; Figure 2). GRO-impregnated panels appeared to be as effective as wicks in affecting male mating performance. In this experiment, control *tsl* males accounted for a similar number of matings per replicate as Maui males, and treated *tsl* males actually obtained more copulations per replicate than Maui males (Table 2). Correspondingly, treated *tsl* males obtained a significantly higher proportion of total matings than control *tsl* males (Figure 2).

The final experiment of 2005, which compared control *tsl* males to treated males exposed to wicks containing 36 ml of GRO (four sources) or panels containing 40 g of GRO (four sources) provided a direct comparison between the effectiveness of wicks and panels in dispensing GRO. Both wick- and panel-exposed *tsl* males had similar mating success as Maui males, whereas Maui males obtained significantly more matings than control *tsl* males (Table 2). The method of GRO exposure had no effect on the relative mating success of treated *tsl* males, with wick-exposed males achieving 52% of all matings compared to 55% for the panel-exposed males ($t = 0.6$, $P>0.05$; Figure 2).

Discussion

The results described here indicate that, at the proper dose and distribution, GRO can effectively aromatize entire rooms and thereby increase the mating success of large numbers of sterile *C. capitata* males. In the present case, trailers holding ca. 14 million males were exposed to doses of GRO ranging from 9 to 36 ml, and in all cases, save one (18 ml at four sources), the exposed males exhibited higher mating competitiveness than non-exposed males. Considered over all experiments (even that which failed to show an effect), the relative mating success averaged 54% for treated *tsl* males compared to 38% for control *tsl* males, an increase of 42% (16/38). The experiments also revealed that (i) a few sources (four) each with a relatively large amount of GRO (9 ml) can be as effective as many sources (18) each with relatively little GRO (0.5–2.0 ml); (ii) the positive effect of GRO exposure on male mating success was evident 4–5 days following exposure; and (iii) GRO-laden panels were as effective as oil-laden wicks in increasing male mating success.

Although the data define clear trends, weaknesses inherent in our bioassay confound predictions regarding the effectiveness of GRO-exposed, sterile males in a large-scale SIT program. Most importantly, the use of field tents bypasses all non-reproductive activities demanded of released, sterile males (foraging and predator evasion) as well as several key reproductive activities, such as the need to locate male mating aggregations (leks), defend leaf territories against conspecific males, and attract females over long distances via pheromone signaling. Thus, while females clearly appear to select GRO-exposed males once within a mating aggregation, it is not known whether GRO-exposed males are capable in the field of completing the behavioral 'hurdles' necessary to gain this close-range advantage. Obviously, GRO-exposed males will not enjoy increased mating success if, for example, they are highly susceptible to predation or unable to locate lek sites.

Although the extrapolation of field-tent data to the field is uncertain, there is strong evidence that GRO-exposure improves the effectiveness of large-scale programs in medfly SIT. Most importantly, perhaps, releases of GRO-exposed and non-exposed, sterile *tsl* males in Hawaiian coffee fields showed that induced egg sterility and male mating competitiveness were both significantly higher in the field receiving treated males than the field receiving control males (Shelly et al., 2007). This result, deriving from the open field, is the strongest evidence yet gathered regarding the field effectiveness of GRO-exposed males. It should be noted that, however, while this study documented treatment-related differences in field performance, it did not demonstrate a GRO-mediated improvement (in terms of reduced time or cost) in the suppression or eradication of the wild medfly population, an objective that was beyond the scope of the research. In addition, data (Shelly et al., 2005) from large field enclosures showed that GRO-exposed, sterile *tsl* males induced higher levels of egg sterility than control sterile *tsl* males over a range of overflooding (sterile : wild males) ratios (5 : 1 – 60 : 1). These data also revealed that lower numbers of treated males than control males were required to realize a given level of egg sterility (see also Barry et al., 2003).

The two aforementioned studies provide the most robust support for the use of GRO in medfly SIT, but other studies are important in showing that GRO exposure has no obvious negative side-effects on medfly males. Studies in the laboratory (Levy et al., 2005) and field tents (Shelly et al., 2004) showed no difference in longevity between GRO-exposed and non-exposed sterile *tsl* males. Additionally, a comparison of trap captures (using the male attractant trimedlure) in Florida between aerially released GRO-exposed and non-exposed sterile *tsl* males detected no difference in the total number of males captured, the number of days over which captures were recorded, or the number of different traps from which captures were recorded (as a measure of spatial dispersion; Shelly et al., 2006). In fact, values for each of these parameters were generally greater for treated than control males, indicating

slightly enhanced longevity and dispersal from GRO exposure. Consistent with these data, J Zermeno (pers. comm.) measured travel distances of tethered males on a laboratory flight mill and found no difference between GRO-exposed and non-exposed males.

Moreover, it should be noted that the present study likely underestimates the increased mating success of treated *tsl* males, because the mating trials of necessity used laboratory-adapted strains as 'surrogate' wild flies. Females of such strains are presumably less discriminating than wild females, resulting in greater acceptance of control *tsl* males and a correspondingly inflated measure of their sexual competitiveness. For example, in mating trials conducted in Hawaii using this same *tsl* strain and wild flies, GRO exposure resulted in an increase in relative mating frequency from 25% for control *tsl* males to 51% for treated *tsl* males (Shelly et al., 2004). Thus, while treated *tsl* males attained approximately the same relative level of mating success in the present study and in the Hawaii study (54 vs. 51% total matings, respectively), the control *tsl* males had substantially higher mating success in the present study than in the Hawaii study (38 vs. 25% total matings, respectively). Consequently, GRO exposure improved the mating success of *tsl* males by only 42% in the present study compared to 104% (26/25) in the Hawaii study using wild flies.

In sum, although much of the data showing a beneficial effect of GRO exposure derives from field cage studies, there is evidence from the open field and large field enclosures that indicate pre-release exposure of sterile males to GRO does, in fact, increase the efficacy of the SIT. In addition to being a simple procedure, GRO treatment is inexpensive. Based on recent prices from Citrus and Allied Essences Ltd., 5 kg of GRO costs $66.40 per kilogram (oil plus domestic shipping). Thus, at a total dose of 36 ml (≈ 41 g) per trailer, the cost of GRO exposure is approximately $0.20 per million *tsl* males (cost of 41 g = $2.72, *tsl* males per trailer = 14 million). As the California program pays approximately $180 per million *tsl* pupae (shipping costs included) from Guatemala, the added cost of GRO exposure is negligible ($0.20/$180 = 0.1%). Other supplies (cotton wicks, pipettes, etc.) plus labor would, of course, increase the total cost but only by a small amount. Although the GRO-laden panels were also effective in improving mating success, the production cost ($5/panel) is relatively high. Panels could conceivably be re-used, but this possibility remains unstudied. We recognize that effective GRO doses may vary among emergence facilities (with the size of the holding rooms used) and consequently believe it unwise to prescribe a generic GRO dose-distribution protocol or predict a generalized estimate of the associated costs.

In conclusion, our research in this area has focused on documenting the effects of GRO exposure and has largely ignored mechanistic questions. Studies conducted in a large field enclosure (Shelly, 2001) and in laboratory wind-tunnel (Papadopoulos et al., 2006) suggest that GRO exposure does not affect the attractiveness of the male sex pheromone over distances of 1 to several meters. Instead, two preliminary findings from ongoing work indicate that GRO aroma interacts with the male exoskeleton in some way to produce a scent attractive to females. First, medfly males exposed to GRO aroma for 30 s immediately before mating trials had a mating advantage over control males. Given the short interval between GRO exposure and testing, it appears unlikely that the increased success of treated males required incorporation and physiological processing of airborne chemicals. Second, in screen cages in the laboratory, females preferentially land on chilled (dead) males that had been exposed (while alive) to GRO the previous day compared to chilled (dead) non-exposed males. The same result was obtained whether the males were visible to females or were covered by a cotton cloth (blocking visual but not olfactory stimuli). Because the males were not moving or behaving in any way, GRO exposure appears to be the only factor responsible for the observed female preference. Future work will include chemical analyses of the cuticular compounds of GRO-exposed vs. non-exposed males.

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